

Supplementary Information for:

Steviol Rebaudiosides bind to four different sites of the human sweet taste receptor (T1R2/T1R3) complex explaining confusing experiments

Shuang Hao¹, Brian Guthrie², Soo-Kyung Kim³, Sergej Balanda⁴, Jan Kubicek⁴, Babar Murtaza⁵, Naim A. Khan⁵, Pouyan Khakbaz², Judith Su^{1,*}, William A. Goddard III^{3,*}

¹College of Optical Sciences, The University of Arizona, Tucson, AZ 85721, USA

²Global Core Research and Development Group, Cargill, Inc. 14800 28th Avenue N. Plymouth, MN 55447, USA

³Materials and Process Simulation Center (MSC), California Institute of Technology, Pasadena, California 91125, USA

⁴Cube-biotech, Creative Campus Monheim, Creative-Campus-Allee 12, 40789 Monheim, Germany

⁵Physiologie de la Nutrition and Toxicologie, UMR U866 Institut National de la Santé et de la Recherche Médicale, Université de Bourgogne-Franche Compté, Agro-Sup Dijon, France

*Corresponding authors: wag@caltech.edu; judith@arizona.edu

ORCID: WAG (0000-0003-0097-5716), SKK (0000-0002-4498-5441), BDG (0000-0002-3508-4625), JS (0000-0002-1005-1755), SH (0000-0002-6153-2401)

Table S1. Competition binding experiments of various ligands with [³H]-Rebaudioside B. The experiments were performed in triplicate, and the error bars represent the standard deviation.

Ligand (reported binding location)	Binding curve	K _i (M) versus [³ H]-Rebaudioside B (presumed VFD2 binding site)
RebM (VFD2)	Competitive binding Reb-m	ambiguous
RebC (VFD2)	Competitive binding Reb-c	0.01899
Ace-K (VFD2)	Competitive binding AceK	1.096

S819 (TMD2)	Competitive binding S819	ambiguous
NHDC (TMD3)	Competitive binding NHDC	0.06337
Amiloride HLC (TMD2)	Competitive binding amiloride HCL	0.001352

Table S2. Competition binding experiments using [^3H]-Lactisole as radioligand against various ligands. The experiments were performed in triplicate, and the error bars represent the standard deviation.

Ligand (reported binding location)	Binding curve	Ki(M) versus [^3H]-lactisole (TMD3 binding site)
RebM (VFD2)	Competitive binding reb-m	0.01175
RebC (VFD2)	Competitive binding reb-c	0.006978
Ace-K (VFD2)	Competitive binding AceK	ambiguous
S819 (TMD2)	Competitive binding S819	ambiguous

NHDC (TMD3)	Competitive binding NHDC <table><caption>Estimated data points from the graph</caption><thead><tr><th>Concentration (log molar)</th><th>DPM</th></tr></thead><tbody><tr><td>10⁻⁸</td><td>~370</td></tr><tr><td>10^{-7.5}</td><td>~390</td></tr><tr><td>10⁻⁷</td><td>~340</td></tr><tr><td>10^{-6.5}</td><td>~360</td></tr><tr><td>10⁻⁶</td><td>~340</td></tr><tr><td>10^{-5.5}</td><td>~240</td></tr><tr><td>10⁻⁵</td><td>~230</td></tr><tr><td>10^{-4.5}</td><td>~240</td></tr></tbody></table>	Concentration (log molar)	DPM	10 ⁻⁸	~370	10 ^{-7.5}	~390	10 ⁻⁷	~340	10 ^{-6.5}	~360	10 ⁻⁶	~340	10 ^{-5.5}	~240	10 ⁻⁵	~230	10 ^{-4.5}	~240	0.0003337
Concentration (log molar)	DPM																			
10 ⁻⁸	~370																			
10 ^{-7.5}	~390																			
10 ⁻⁷	~340																			
10 ^{-6.5}	~360																			
10 ⁻⁶	~340																			
10 ^{-5.5}	~240																			
10 ⁻⁵	~230																			
10 ^{-4.5}	~240																			

Table S3. Competition binding experiments using [³H]-Perillartine as radioligand. The experiments were performed in triplicate, and the error bars represent the standard deviation.

Ligand (reported binding location)	Binding curve	Ki(M) versus [³ H]-Perillartine (TMD2 binding site)
RebM (VFD2)	Competitive binding reb-m	ambiguous
RebC (VFD2)	Competitive binding reb-c	ambiguous
Ace-K (VFD3)	Competitive binding AceK	0.1420
S819(TMD2)	Competitive binding S819	ambiguous

NHDC (TMD3)	Competitive binding NHDC DPM Concentration (log molar)	0.2937
Amiloride HCL (TMD2)	Competitive binding amiloride HCL DPM Concentration (log molar)	ambiguous

Table S4. The free energy of the binding of steviol glycosides to heterodimeric sweet taste receptor (T1R2/T1R3) using molecular mechanics with generalized Born and surface area solvation (MM/GBSA).

site	Title	MMGBSA dG Bind	delta VFD2		MMGBSA dG Bind	delta VFD2		MMGBSA dG Bind	delta VFD2
1	RebB	14.56	46	Rubu	-51.54	22.3	RebC	8.31	52.03
2	RebB	-45.65	-14.21	Rubu	-41.43	32.41	RebC	-59.03	-15.31
3				Rubu	-45.84	28	RebC	-41.11	2.61
4	RebB	-22.13	9.31	Rubu	-48.17	25.67	RebC	-26.38	17.34
5	RebB	-31.44	0	Rubu	-73.84	0	RebC	-43.72	0
6	RebB	-39.83	-8.39	Rubu	-36.09	37.75	RebC	-54.95	-11.23
8	RebB	-18.21	13.23	Rubu	-40.61	33.23	RebC	-68.16	-24.44
9	RebB	-21.93	9.51	Rubu	-27.41	46.43	RebC	-33.85	9.87
10	RebB-m	-37.79	-6.35				RebC	-49.19	-5.47

Table S5. The disulfide bond comparison between our predicted structure and the AlphaFold predicted structure for the hetrodimeric sweet taste receptor (T1R2/T1R3).

TAS1R	Domain	Disulfide bridge	Gensembel-MD	AlphaFold3
1R2	VFD	C59-C102	O	O
		C359-C363	O	X
		C405-C410	O	O
	VFD-CRD	C233-C513	O	O
	CRD	C495-C514	O	O
		C499-C517	O	O
		C520-C535	O	O
		C538-C551	O	O
	TMD	C630-C720	O	O
1R3	VFD	C62-C103	O	O
		C351-C370	O	X
		C370-C373	X	O
		C373-C375	O	X
		C410-C415	O	O
	VFD-CRD	C236-C517	O	O
	CRD	C499-C518	O	O
		C503-C521	O	O
		C524-C538	O	O
		C541-C554	O	O
	TMD	C605-C694	O	O
1R2-1R3	VFD	C359-C129	X	O

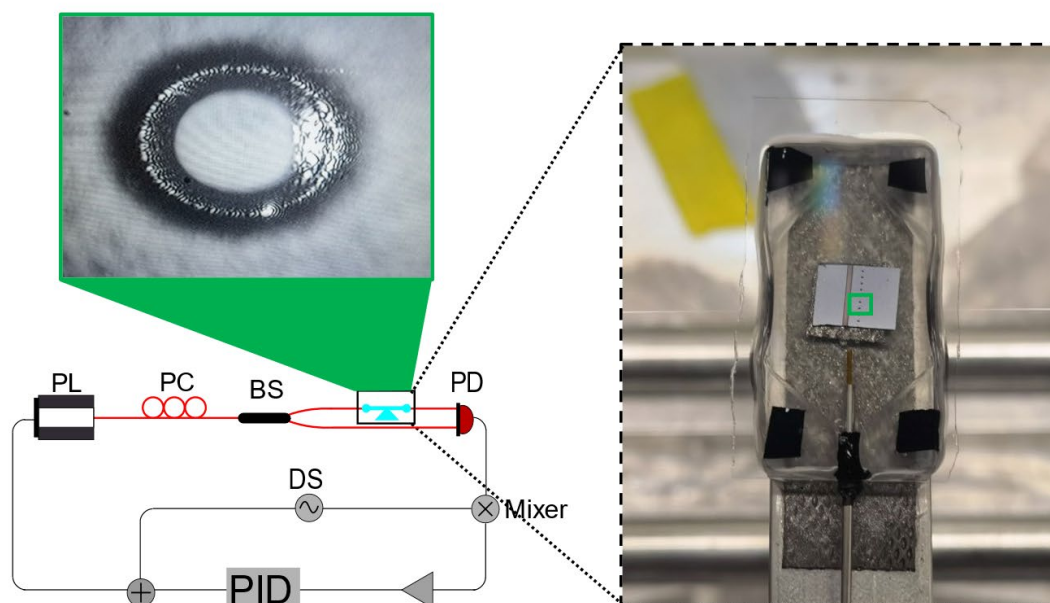


Figure S1. Overview of Frequency Locked Optical Whispering Resonator (FLOWER). A schematic diagram of FLOWER is shown (bottom left). A 2 kHz oscillation dither signal is utilized to modulate the laser frequency. A polarization controller (PC) allows for precise control of the probe laser's (PL) polarization. A 50:50 fiber-coupled beam splitter (BS) splits the light into the signal and reference arms of an auto-balanced receiver. A proportional-integral-derivative (PID) controller is employed to set the laser frequency to the microcavity resonance, guided by the received error signal. The resonance shift is acquired by continuously tracking the laser wavelength through the PID controller output. The top left figure illustrates the microtoroid coupled to the tapered fiber in an aqueous environment. The right inset shows the fluidic chamber. A 100 μm diameter needle is used for injecting the ligand solution.

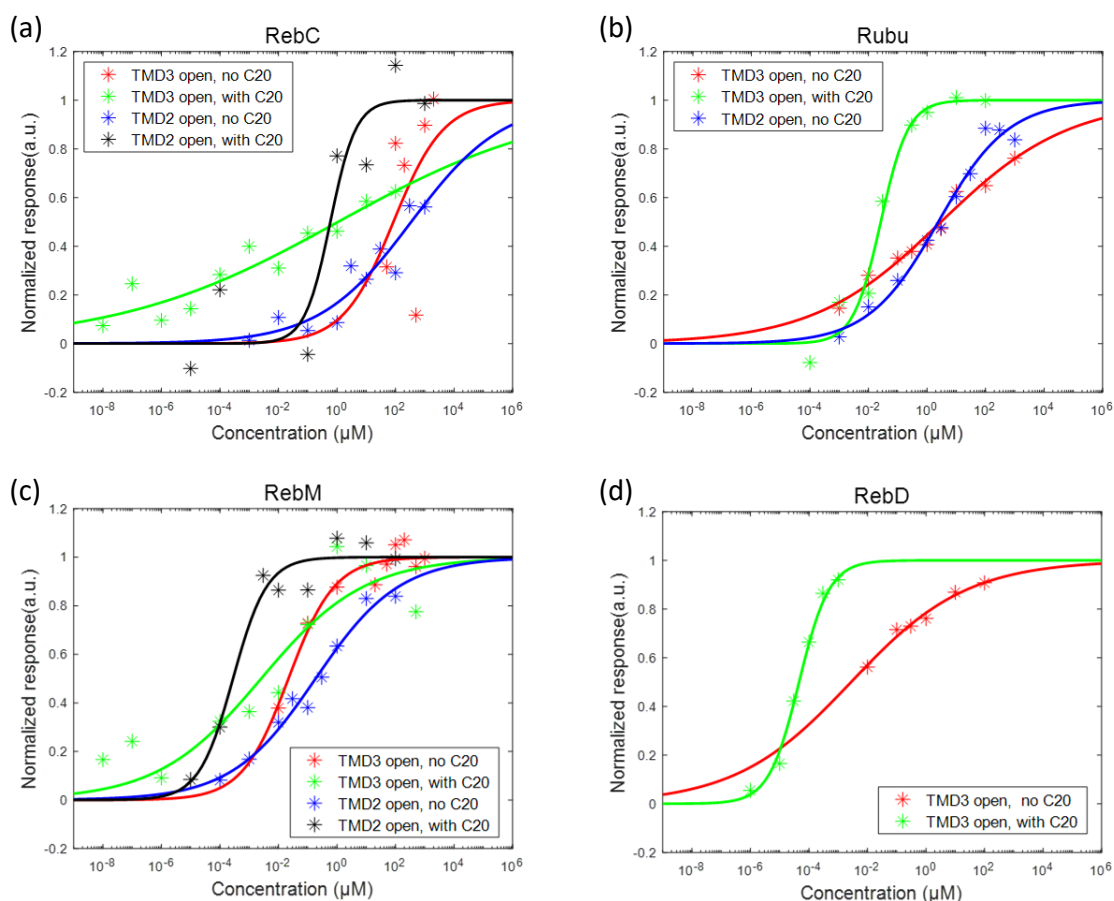


Figure S2. Normalized binding response curve of various sweet ligands binding to the human sweet receptor T1R2/T1R3 for various conditions. (a) Binding of RebC. (b) Binding of Rubu. (c) Binding of RebM. (d) Binding of RebD. We note that some of the binding curves for the no C20 condition did not reach saturation. This indeed affects the binding affinity and Hill slope measurements from the binding curve. In the absence of C20, the binding affinity of steviol glycosides to the sweet taste receptor T1R2/T1R3 is weakened, resulting in higher K_d values. Additionally, the high concentration of steviol glycosides required to reach saturation is limited by their solubility in the experimental HEN buffer. Therefore, under the no C20 condition, some binding data did not reach saturation due to a combination of higher K_d values and low solubility in the experimental buffer. We had to use the limited concentration sweet ligand binding response to perform the curve fitting. We further note that the RebD binding curves shown here are normalized responses, where the binding curve starts from a baseline at zero and reaches saturation near 1. The binding curve of RebD without C20 is not forced to match the baseline of RebD with C20. Each curve is fit separately, and both demonstrate good fitting results. In the curve for RebD without C20, the fitted baseline is lower than the response at the minimum concentration. In the curve for RebD with C20, the baseline is near the minimum concentration response. Despite these differences, the RebD curves show good fitting results, with R^2 values of 0.97 (no C20) and 0.98 (with C20), respectively.

Table S6. The coefficient of determination R^2 for the binding response curves in Figure S2

T1R2/T1R3	RebC	Rubusoside	RebM	RebD
TMD2 open (No C20)	0.97	0.95	0.98	
TMD3 open (No C20)	0.73	0.97	0.79	0.97
TMD2 open (+C20)	0.92		0.97	
TMD3 open (+C20)	0.90	0.95	0.80	0.98

[³H]-Rebaudioside B: association, then dissociation

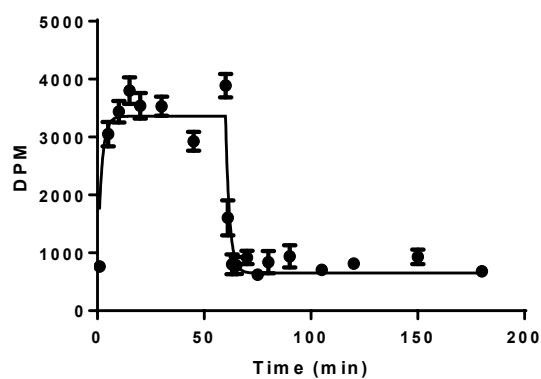


Figure S3. Kinetic binding using [³H]-Rebaudioside B as radioligand.

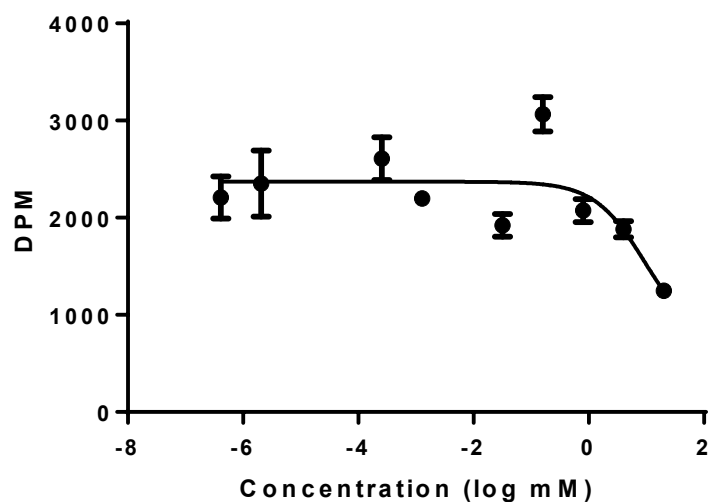


Figure S4. Effect of GDP on the binding of [³H]-Rebaudioside B

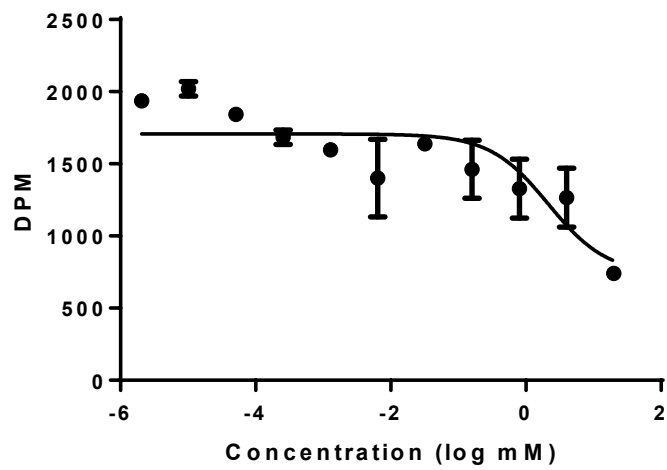


Figure S5. Effect of GTP on the binding of $[^3\text{H}]$ -Rebaudioside B

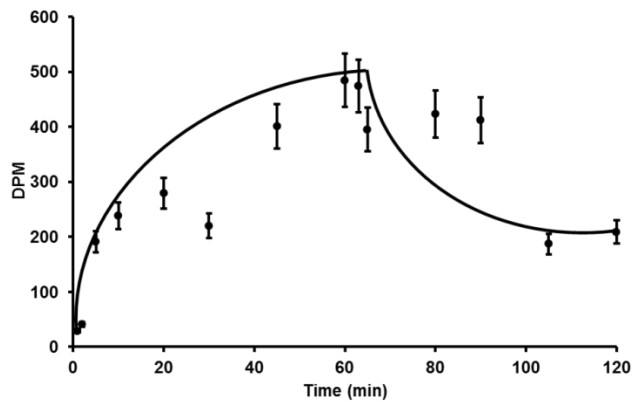


Figure S6. Kinetic binding using $[^3\text{H}]$ -Lactisole as radioligand

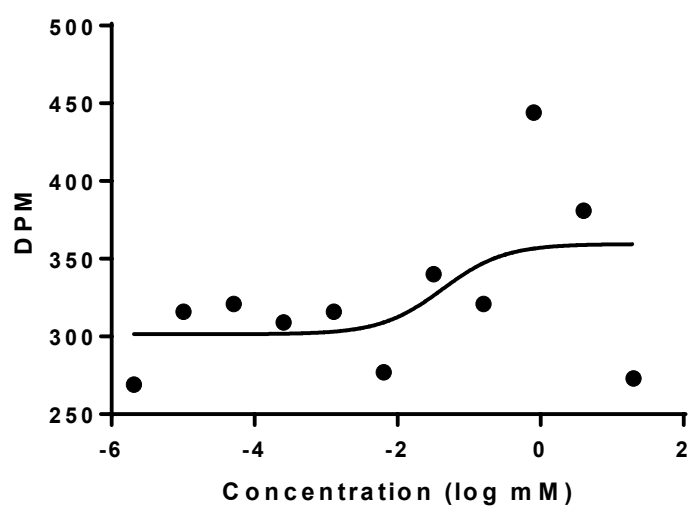


Figure S7. Effect of GDP on the binding of $[^3\text{H}]$ -Lactisole

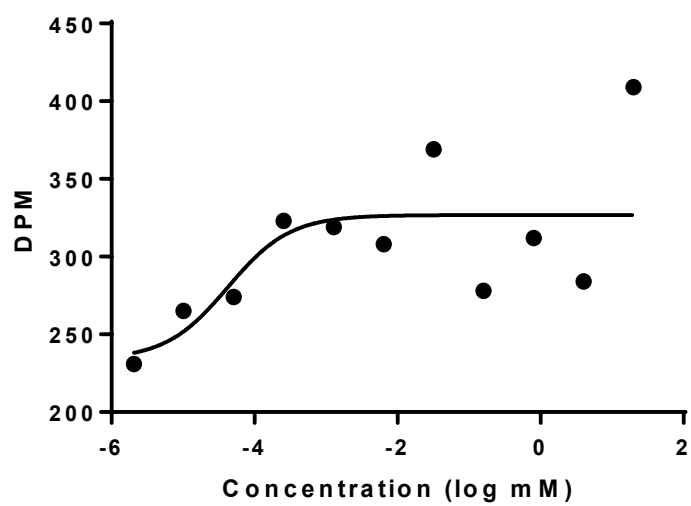


Figure S8. Effect of GTP on the binding of $[^3\text{H}]$ -Lactisole

[³H]-Perillartine: association, then dissociation

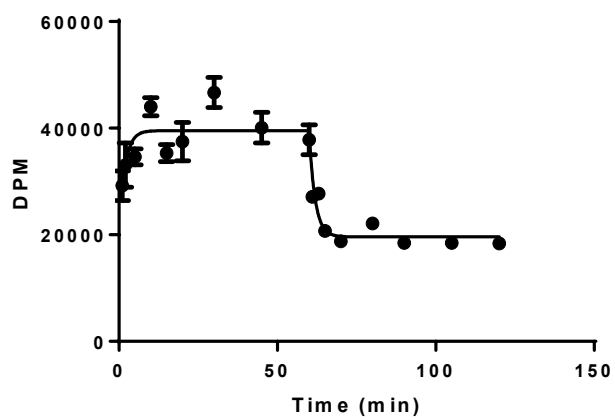


Figure S9. Kinetic binding using [³H]-Perillartine as radioligand

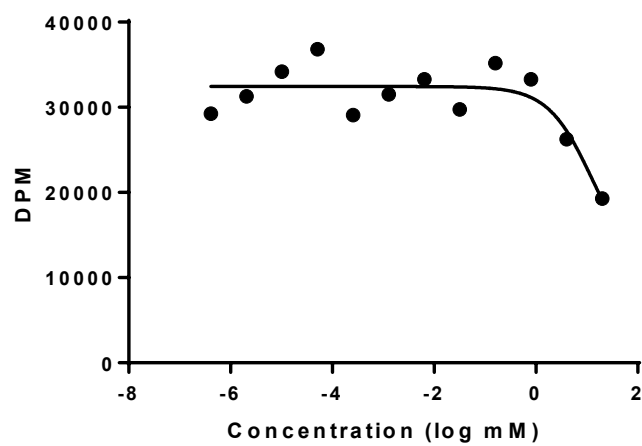


Figure S10. Effect of GDP on the binding of [³H]-Perillartine

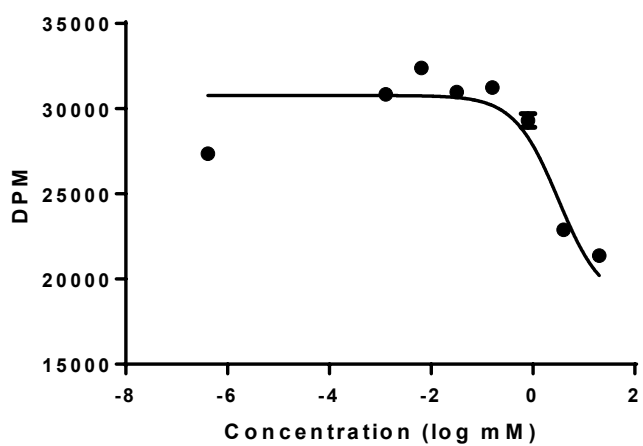


Figure S11. Effect of GTP on the binding of [^3H]-Perillartine

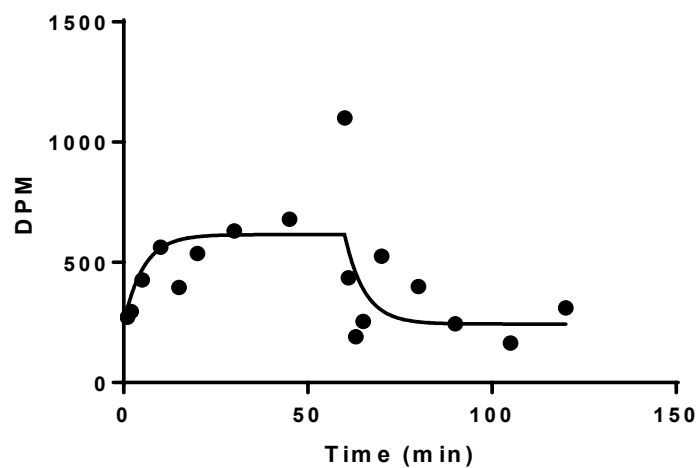


Figure S12. Kinetic binding using [^{14}C]-sucrose as radioligand.

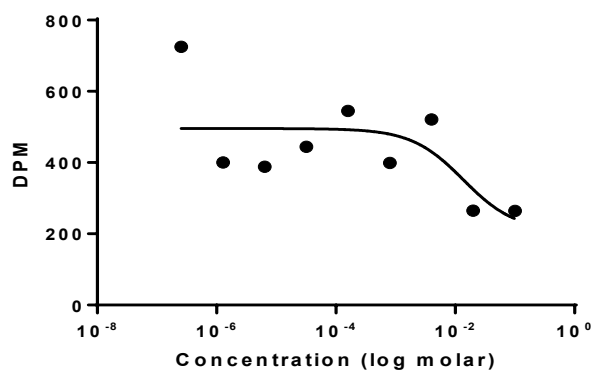
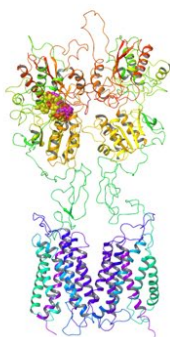


Figure S13. Competition binding curve of variable concentration of NHDC against fixed concentration of [¹⁴C]-sucrose.

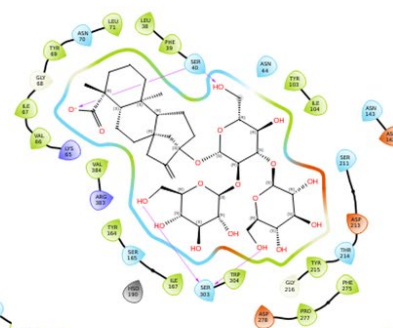
Site5, CO, VFD2



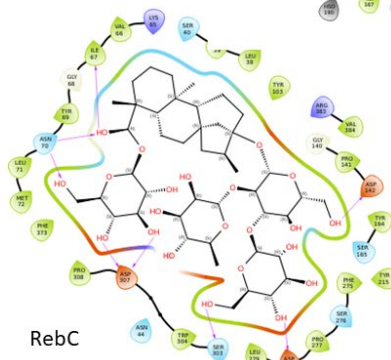
High sweet:

Low sweet:

RebB



RebC

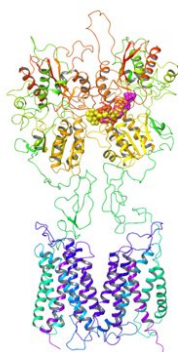


Rubu



Rubu=pink, rebc=yellow, rebM=green, rebD=blue

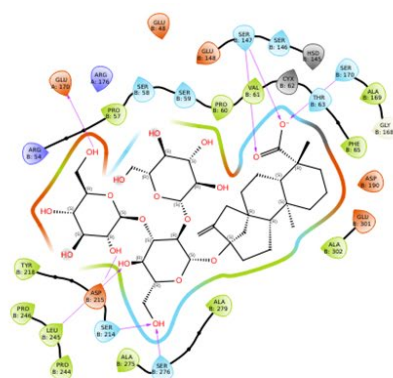
Site1: CO, VFD3



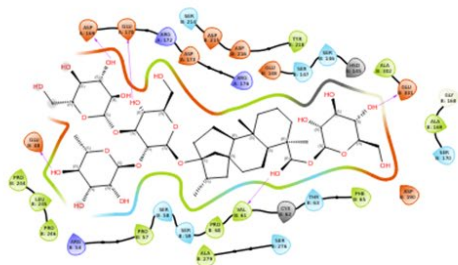
High sweet:

Low sweet:

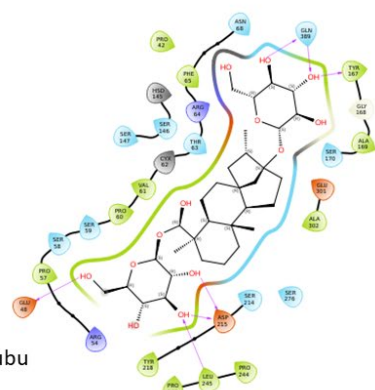
RebB



RebC

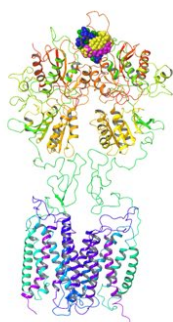


Rubu



Rubu=pink, rebc=yellow, rebB=orange

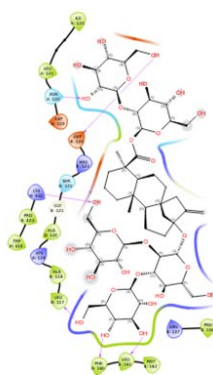
Site4 CO-VFD3



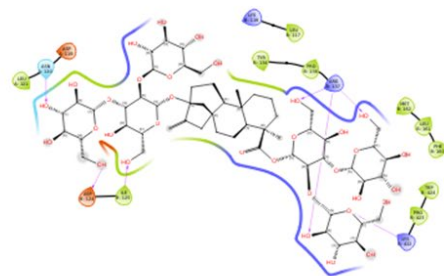
High sweet:

Low sweet:

RebD



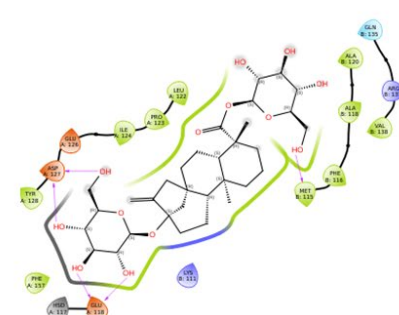
RebM



RebC

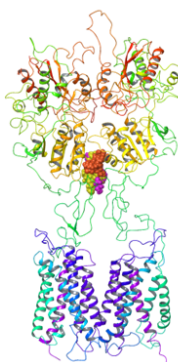


Rubu



Rubu=pink, rebc=yellow, rebM=green, rebD=blue

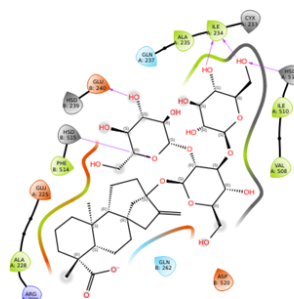
Site6:CRD -2-3



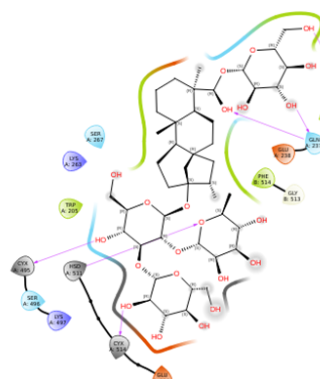
High sweet:

Low sweet:

RebB



RebC

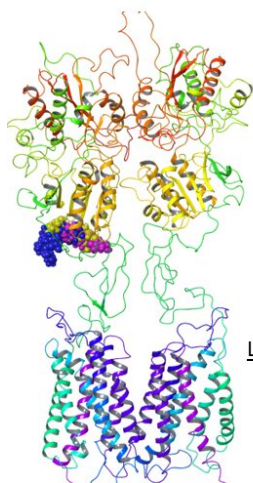


Rubu



Rubu=pink, rebc=yellow, rebB=orange

Site9:VFD-2-lower

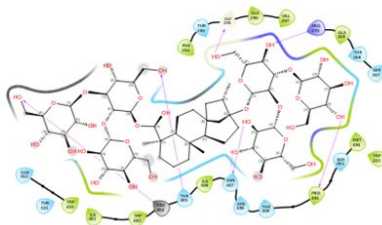


High sweet:

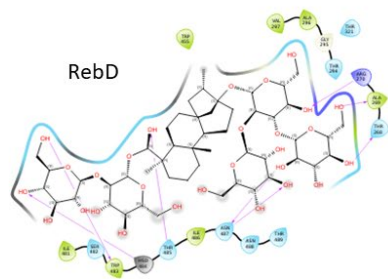
Low sweet:

Rubu=pink, rebc=yellow, rebB=orange

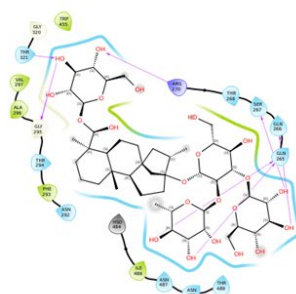
RebM



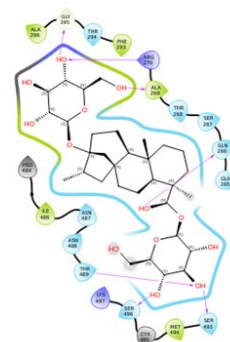
RebD



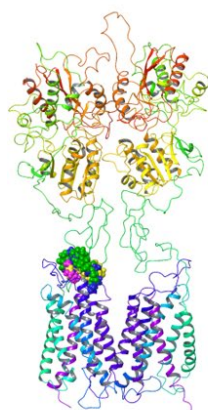
RebC



Rubu



Site10:TMD2

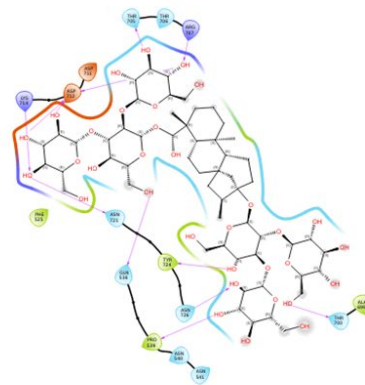


High sweet:

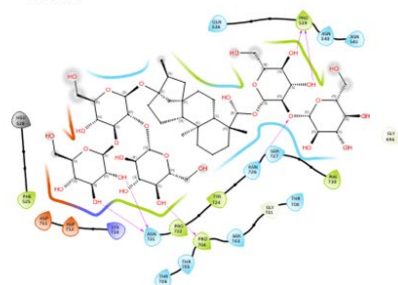
Low sweet:

Rubu=pink, rebc=yellow, rebB=orange

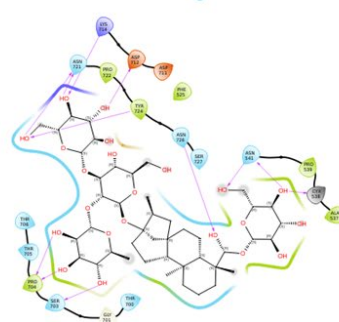
RebM



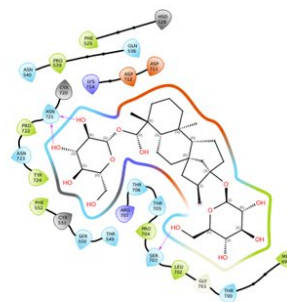
RebD



RebC



Rubu



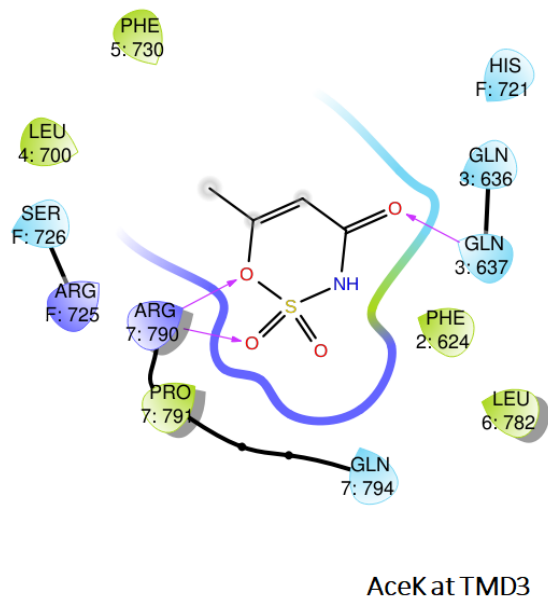
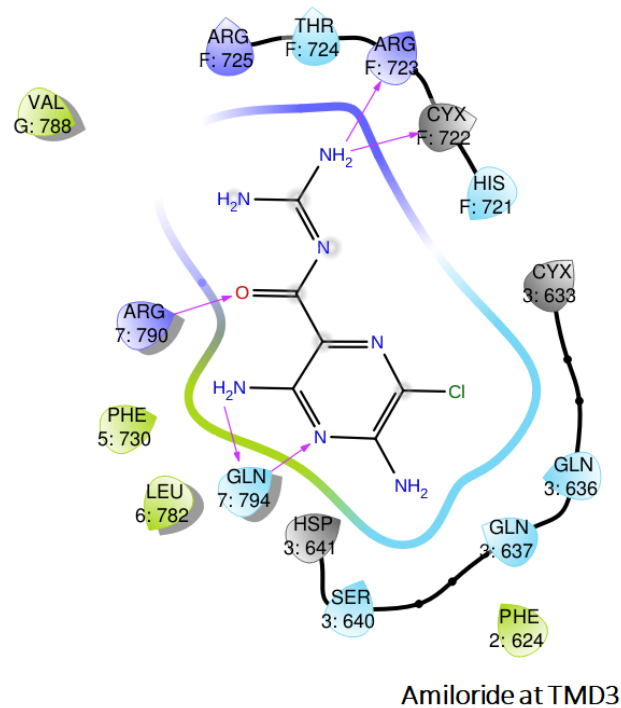
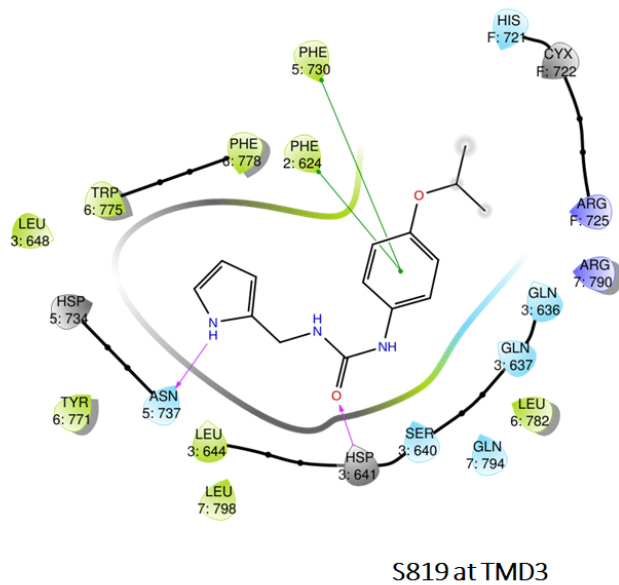
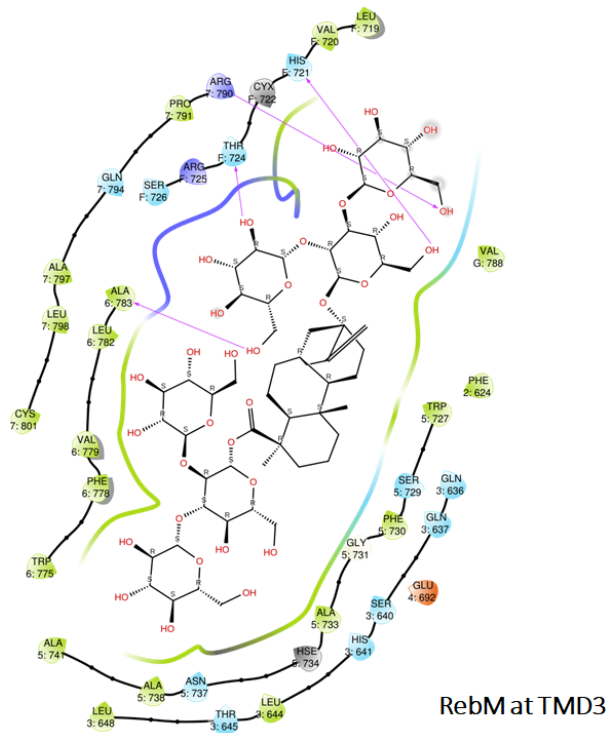


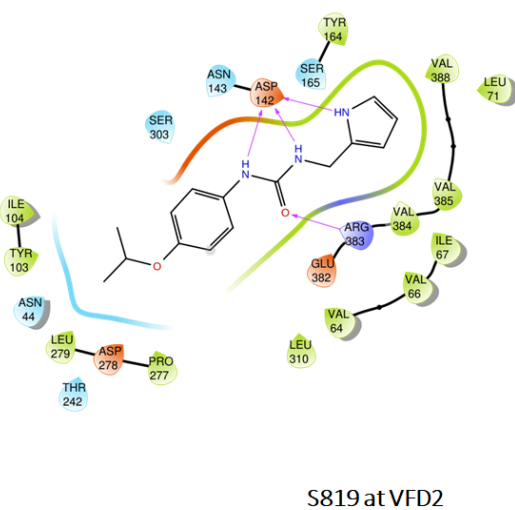
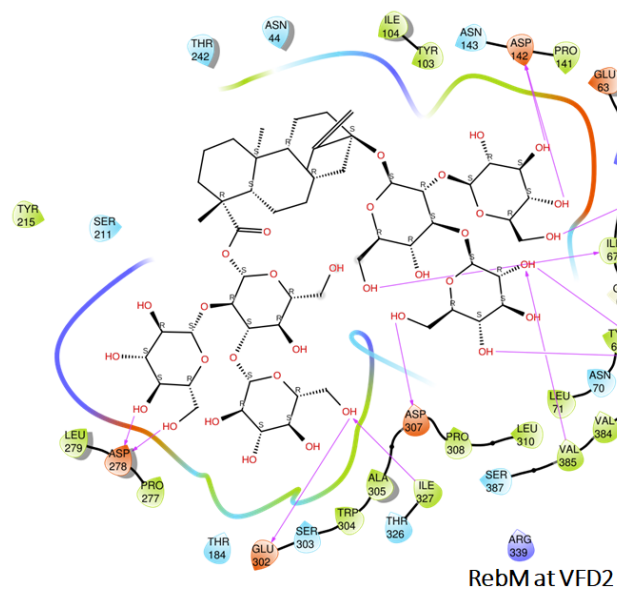
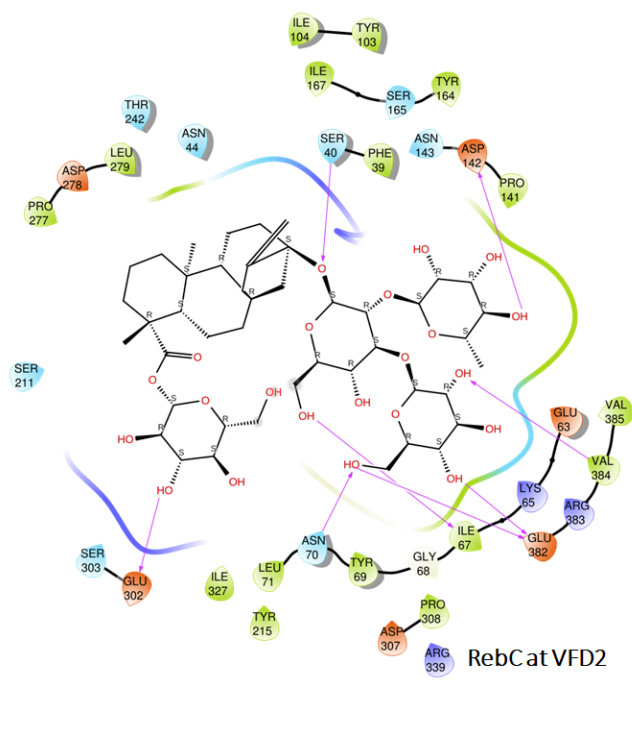
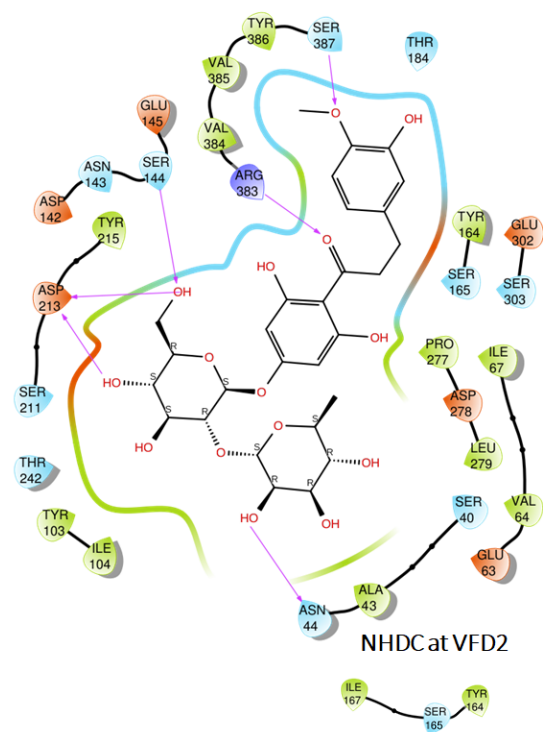
RebD

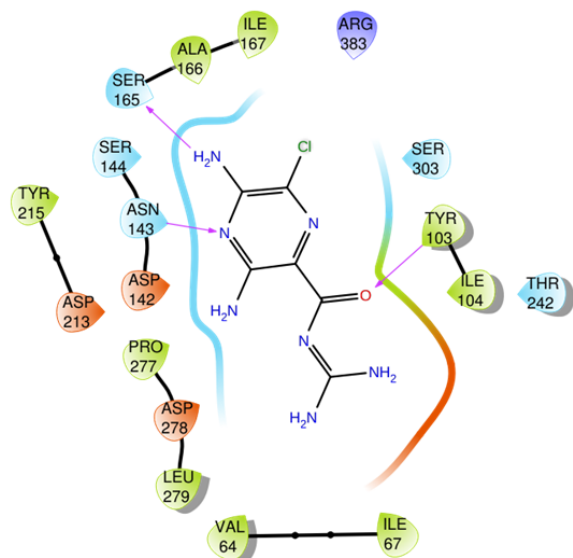
RebC

Rubu

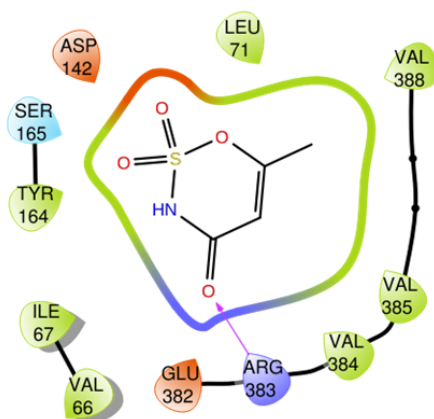
20



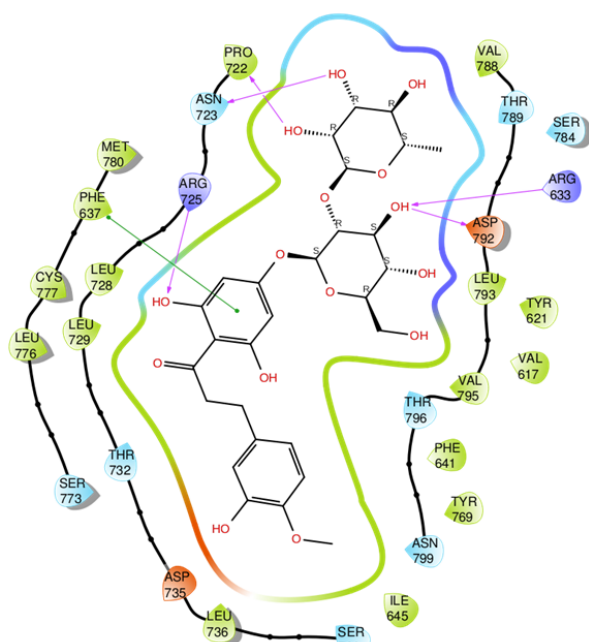




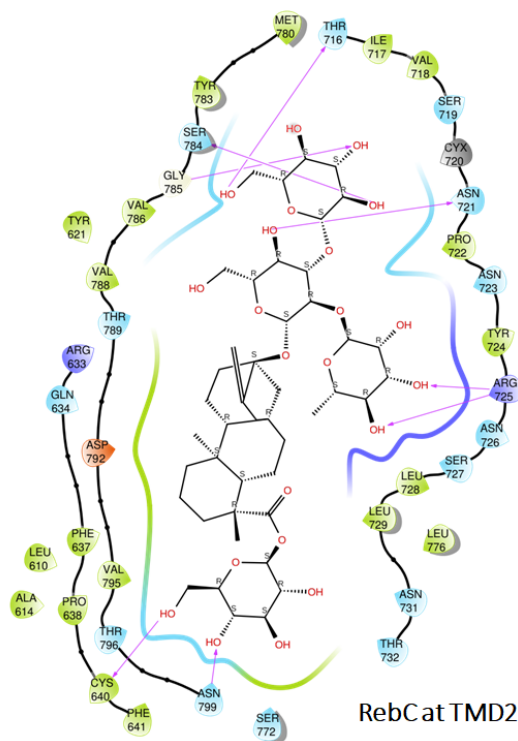
Amiloride at VFD2



AceK at VFD2



NHDC at TMD2



RebCat TMD2

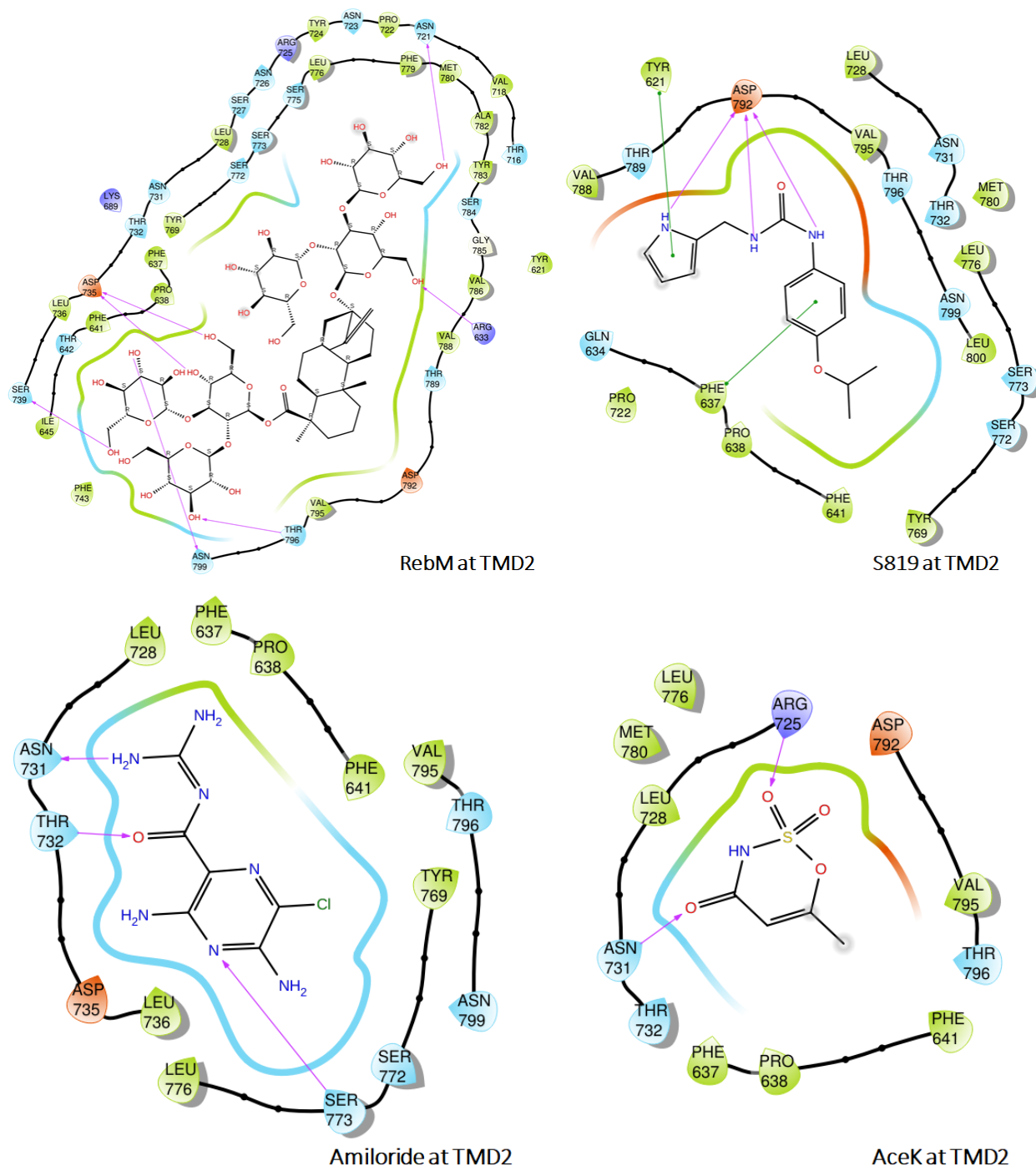


Figure S15. Ligand interaction diagram for various steviol glycosides (Rebaudioside B (RebB), RebM, RebC), artificial sweeteners (neohesperidin dihydrochalcone (NHDC), acesulfame K (AceK), amiloride), and a positive allosteric modulator (S819) at distinct binding sites: VFD2 (VFD of 1R2), TMD2, and TMD3. Hydrogen bonds are indicated by arrows.

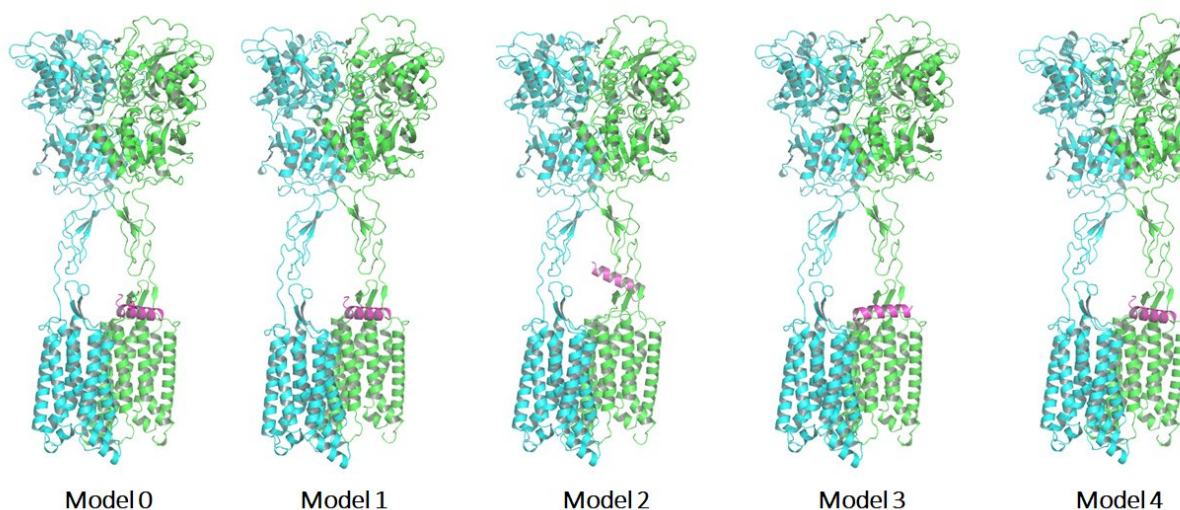


Figure S16. Five models of the sweet taste receptors, T1R2 (Green)/ 1R3 (Cyan) with C20 (Pink) from AlphaFold prediction.

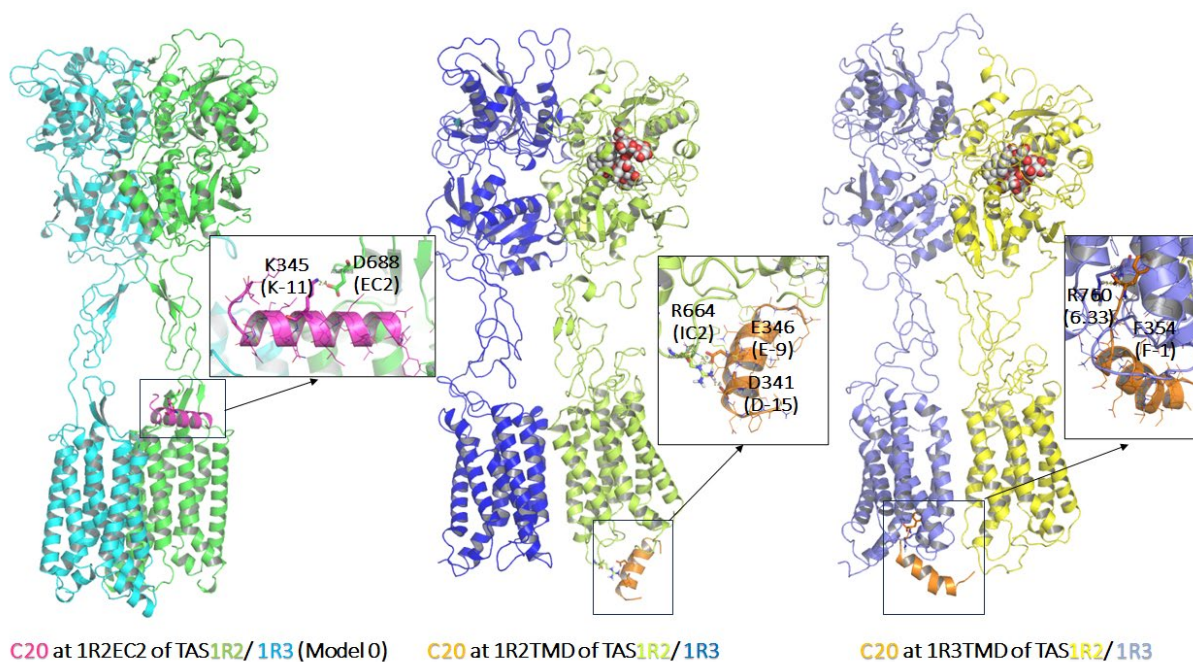


Figure S17. The overall structures of model 0 of T1R2 (Green)/ 1R3 (Cyan) with C20 (Pink) from AlphaFold prediction, and our MD structures of RebM-bound TAS1R2 (Yellow) /1R3 (Blue) with C20 (Orange) at T1R2TMD or T1R3TMD.